PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference SCB/P60770/002	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/04950	International filing date *(day/month/year)* 13.11.2003	Priority date *(day/month/year)* 14.11.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/574, G01N33/564		
Applicant THE UNIVERSITY OF NOTTINGHAM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.06.2004	11.01.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Vanhalst, K Telephone No. +31 70 340-3075

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/04950

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-39	as originally filed

Claims, Numbers

1-32, 34, 36-38	as originally filed
33, 35	received on 25.10.2004 with letter of 25.10.2004

Drawings, Sheets

1/12-12/12	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	11,12,20-38
	No:	Claims	1-10,13-19
Inventive step (IS)	Yes:	Claims	11,12,20-24,30,32-38
	No:	Claims	1-10,13-19,25-29,31
Industrial applicability (IA)	Yes:	Claims	1-38
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Cited documents

D1: WO 99/58978 A (PRICE MICHAEL RAWLING ;UNIV NOTTINGHAM (GB); GRAVES CATHERINE ROSA) 18 November 1999 (1999-11-18)

D2: LUO L -Y ET AL: "Identification of heat shock protein 90 and other proteins as tumour antigens by serological screening of an ovarian carcinoma expression library" BRITISH JOURNAL OF CANCER, vol. 87, no. 3, 29 July 2002 (2002-07-29), pages 339-343, XP001189013 ISSN: 0007-0920

D3: US-A-5 747 268 (HERRING KATHRYN ET AL) 5 May 1998 (1998-05-05)

D4: WOLF A ET AL: "AN IMPROVED ANTIGENIC MARKER OF HUMAN LUNG CARCINOMAS AND ITS USE IN RADIO IMMUNOASSAYS" BRITISH JOURNAL OF CANCER, vol. 43, no. 3, 1981, pages 267-275, XP008028512 ISSN: 0007-0920

2 **Novelty** (Art. 33(2)) PCT

2.1 D1 discloses (the references in parentheses refer to these documents):

2.1.1 An immunoassay method of detecting cancer-associated anti-tumour auto-antibodies (abstract of D1), wherein the immuno-reagent comprises one or two or more tumour marker proteins, prepared from (p8, lines 22-27 of D1) bodily fluids (defined on p6, lines 7-9 of D1 as: plasma, serum, blood, urine, sweat, lymph, faeces, cerebrospinal fluid or nipple aspirate) of one or more cancer patients (p6, lines 1-6; p14, lines 17-25), in which the tumour marker proteins exhibit selective reactivity (affinity is 3-6 times greater than that of the non-tumour antigen, cf. claim 35 of D1) for the cancer associated anti-tumour auto-antibodies. D1 thus discloses all features of claims 1,2 and 13.

2.1.2 The use of tumour markers, selected from MUC-1, MUC-16 or c-myc, c-erbB2, p53, ras, BRCA1, BRCA2, APC, PSA, CEA and CA19.9 (D1: p8, lines 1- 26), as stated in claims 15 and 17 respectively.

2.1.3 The different uses of the method of 2.1.1 (D1: p7, line 1 - p9, line 34; examples 1-15; claims 1-72), as stated in claims 3-10, 12,14 and 18.

2.1.4 The use of tumour marker protein prepared from an excretion of one or more cancer patients, in the manufacturing of an immunoassay reagent exhibiting selective reactivity for cancer-associated anti-tumour auto-antibodies c-erbB2, p53, ras, BRCA1, BRCA2, APC, PSA, CEA and CA19.9 (D1: abstract; p7, line 1 - p9, line 34; examples 1-15; claims 1-72), as stated in claim 19.

2.2 In conclusion, the subject-matter of claims 1-10,13-19 is not new and the application does therefore not fulfill the requirements of Article 33(2) PCT.

2.3 The combination of the features in the subject-matter of claims 11,12,20-38 was not found in the cited prior art documents. Claims 11,12,20-38 is therefore new.

3 **Inventive step** (Art. 33(3) PCT)

3.1 D1 is regarded as being the closest prior art regarding the subject-matter of claims 11,12,20-24,32-38, and discloses a method as described in 2.1.1 (cf. D1: abstract; p7, line 1 - p9, line 34; examples 1-15; claims 1-72), which differs from the subject-matter of claims 11,12,20-24,32-38 in that said tumour-associated antigens are isolated form bodily fluids, derived from a cavity or space wherein a tumour is or was present. From the examples in the present application follows that the reactivity of tumour-marker proteins isolated from e.g. pleural effusions from a cancer patient, in an immunoassay to detect auto-antibodies, is similar to that of tumour-marker proteins isolated from serum of a cancer patient (description p32, lines 24-31). The only technical effect that could be identified is that a higher yield of tumour-specific antigen-variants can be isolated from e.g. pleural effusions.

3.1.1 The objective problem can therefore be regarded as: "How to provide a method to isolate a higher yield of tumour-marker proteins from a cancer patient?". The solution would then be to use bodily fluids, derived from a cavity or space wherein a tumour is or was present, as a source to isolate tumour-marker proteins.

3.1.2 None of the prior art documents cited in the international search report indicate the advantage to use bodily fluids, derived from a cavity or space wherein a tumour is or was present, for the isolation of tumour-marker proteins. The artisan, in search for a method to increase the yield of the tumour-marker protein isolation method, would not find it obvious to use bodily fluids, derived from a cavity or space wherein a tumour is or was present, as a source.

3.1.3 The subject-matter of **claims 11,12,20-24,32-38** can therefore be regarded as comprising an inventive step, fulfilling the requirements of Article 33(3) PCT.

3.2 D1 which is regarded as the closest prior art regarding the subject-matter of claims 25-31 discloses a method of preparing a tumour-marker protein, from which the subject-matter of claims 25-31 differs in that the tumour-marker protein is isolated out of a pool of excretions from two or more patients in stead of serum of one or more cancer patients (cf. D1: p14, lines 17-24). Their is no technical effect due to this difference, since the present application does not show the use of any of the excretions in the isolation method.

3.2.1 The objective problem can therefore be regarded as: "How to provide an alternative method to isolate tumour-marker proteins form one or more patients". The solution being the isolation of tumour-marker proteins out of a pool of secretions from two or more patients.

3.2.2 Although D1 does not explicitly disclose the isolation of tumour-marker proteins out of a pool of excretions from two or more patients in the examples, it clearly suggests that tumour-marker proteins could be isolated "and thus pooled" from serum of one or more patients (cf. D1: p14, lines 17-24). Furthermore, D1 generally discloses the isolation of tumour-marker proteins from bodily fluids (cf. D1: p7, lines 9-20; p, lines 22-27), which are defined as plasma, serum, whole blood, urine, sweat, lymph, faeces, cerebrospinal fluid or nipple aspirate (cf D1: p6, lines 6-9). This clearly indicates that serum on the one hand and excretions such as urine, sweat or feaces on the other hand could easily be interchanged in the method of D1. Since there is no indication of differences in the immunogenic activities of tumour-marker proteins isolated from a pool of urine

of cancer patients or from a pool of serum of cancer patients, the use of excretions such as urine, sweat or feaces in the method of D1, would be an obvious choice for the person skilled in the art.

3.2.3 The subject-matter of **claims 25-29,31** can therefore not be seen as comprising an inventive step and the application does therefore not fulfil the requirements of Article 33(3) PCT.

3.3 D1 which is regarded as the closest prior art regarding the subject-matter of **claim 30**discloses a method of preparing a tumour-marker protein, from which the subject-matter of claim 30 differs in that the method includes a step of removing contaminating immunoglobulins from the tumour-marker preparation. The present application remains silent regarding the technical effect due to this difference.

3.3.1 The objective problem can therefore be regarded as: "How to provide an alternative method to isolate tumour-marker proteins form one or more patients". The solution being the removal of contaminating immunoglobulins from the tumour-marker preparation.

3.3.2 Although removal of immunoglobulins of a tumour-marker sample is a routine procedure, none of the prior art documents cited in the search report discloses or suggests this procedure. It would therefore not be obvious for the artisan to incorporate this step in the method of D1.

3.3.3 The subject-matter of **claim 30** can therefore be seen as comprising an inventive step, required by Article 33(3) PCT.

4 Industrial applicability

4.1 The subject-matter of claims 1-38 fulfil the requirements of Article 33(3) regarding industrial applicability.

Re Item VII

Certain defects in the present application

1 Lack of Support

1.1 Claims 1-38 partially refer to the use of excretions such as urine, feaces or seminal fluid as a source for the isolation of tumour-marker proteins. However, there are no examples in the description of the present application indicating that excretions can be used for the isolation of tumour-marker proteins. The present application only discloses the use of urine to isolate "wild-type" antigens as a reference. The present application shows that serum comprises a far lower level of tumour-marker proteins than e.g. pleural effusion fluid. Since blood and therefore also serum is in much closer contact with the tumour tissue than an excretion such as urine or feaces, it is not clear whether such excetions contain sufficient amounts of tumour-specific antigens. It is therefore doubtful whether excretions such as urine or feaces would be suitable as a source of tumour-marker proteins for use as an immunogenic reagent of the present application.

1.2 The subject-matter of claims 1-38 therefore lacks sufficient technical support regarding the use of excretions in the methods of the application. The application does therefore not fulfil the requirements of Article 6 PCT.

and which is substantially immunoglobulin free.

33. A kit or reagent suitable for carrying out an immunoassay which comprises a preparation of a tumour marker protein as claimed in claim 32 immobilized to a solid support.

34. A kit or reagent as claimed in claim 33 wherein said solid support is the surface of a well of a multiwell plate or is a bead.

35. A kit or reagent as claimed in claim 33 or 34 wherein said immobilized tumour marker protein is absorbed, adsorbed or covalently attached to said solid support.

36. Use of a preparation as claimed in claim 32 in the evaluation in an *in vitro* test for the therapeutic efficacy or safety of said tumour marker protein.

37. Use of a preparation as claimed in claim 32 in manufacture of a composition for the evaluation in an *in vivo* test of the therapeutic efficacy or safety of said tumour marker protein.

38. A method of calibrating an assay for measurement or detection of a given tumour marker protein in a clinical sample which method comprises the steps of:

a) preparing at least two samples of a preparation of claim 32, each of which comprises said given tumour marker protein and each of which has a different tumour marker protein concentration to each of the other said samples:

b) carrying out a quantitative measurement of the concentration of said tumour marker protein in each of said samples using

(i) a spectrophotometric method and/or,